immunoglobulin, said transformed yeast useful in the method for production of intact antibodies isolated from DD1 hybridoma (ATCC Accession Number HB9741), in amounts from about 10 to about 36 mg/l in from about 12 to about 108 hours.

REMARKS

This Amendment and Remarks are filed in response to the Final Office Action dated November 15, 2000 wherein pending claims 1-13 and 19-21 stand rejected.

Rejections Under 35 USC 112, Second Paragraph

Examiner maintains his rejections of claims 1-13 under 35 USC 112, second paragraph, as applied to paragraph 10j, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for the reasons set forth in the previous Office Action.

The response has been carefully considered but is deemed not to be persuasive. The response states that claims 6 and 7 have been amended. In response to this argument, it is true that the claims have been amended, but the term "pPICZαLH" was added to claim 1. Thus, claims 1-13 remain indefinite.

In the prior rejection, Examiner argues that the term "pPICZαLH" is indefinite because different laboratories may use different description for the same plasmid or the same description for different plasmid.

Applicants disagree. The term "pPICZαLH", as used herein, is absolutely definite. The term refers to *P. pastoris* yeast

expression vector pPICZαB commercially available and obtained from InVitrogen, Carlsbad, California (see Specification, page 26, lines 31-33). Using this particular expression vector, the plasmid pPICZαLH was constructed according to the Example 2, page 27 and 28. Since the claims are interpreted in the view of the specification, it is impossible that the "pPICZαLH" term would read on other plasmids which would be derived from different organism and contain different DNAs.

However, to overcome the rejection, Claim 1 has been amended to further define the term "pPICZαLH". With this amendment it is believed that the claim 1 and dependent claims 2-13 are definite and the rejection is overcome.

Examiner newly rejects claims 1-13 and 21 under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-13 are indefinite for reciting "selecting an antigen against which the specific antibody is to be produced" in claim 1 because the exact meaning of the phrase is not clear. Does the antibody produced in the method have to be directed against the selected antigen or do you need to just select any antigen?

Applicants disagree. Applicants do not understand Examiner's confusion. The method of the invention is suitable for production of antigen-specific recombinant antibodies (page 1, line 14, for example). Consequently, before beginning of production of such antibodies, one logically selects an antigen against which one to

wish produce the antibodies. Not any antigen but the antigen against which the antibody is desired. In the interest of advancing the prosecution, Applicants amended claim 1 and deleted the language "selecting an antigen against which the specific antibody is to be produced". It is believe that with this amendment, this rejection is overcome.

Claims 5-7 and 21 are indefinite in the recitation of "hybridoma DD1" in claim 5 and 21 because other laboratories/inventors may use the same laboratory designation to refer to different antibodies/hybridomas. Amendment of the claim to insert the corresponding ATCC accession number of the hybridoma which produces the antibody or to add the SEQ ID NOS of the heavy and light chain variable regions would overcome this rejection.

Applicants disagree. However, to meet Examiner's rejections, Applicants amended claims to include the ATCC Accession number.

Claims 6 and 7 are indefinite for reciting "light chain cDNA from the DD1 hybridoma" and "heavy chain cDNA from the DD1 hybridoma" in claim 6 because the exact meaning of the phrases are not clear. It is not clear if the cDNA is from the DNA encoding the antibody produced by hybridoma DD1 or if the cDNA is from the hybridoma cell DD1.

Applicants disagree. Applicants believe that the meaning of the claims 6 and 7 is quite clear but cancelled these claims to meet Examiner's rejections.

Claims 11-13 are indefinite for reciting in claim 11 "origin of the recombinant antibody" because the exact meaning of the

phrase is not clear. Does the phrase mean where the antibody comes from?

Applicants disagree. However, in order to advance the prosecution, Applicants amended claim 11.

With the above described amendments, Applicants believe that all rejections under 35 U.S.C. 112, second paragraph are overcome.

Rejections Under 35 USC 112, First Paragraph

Claims 5-7 and 21 are rejected under 35 USC Section 112, first paragraph, because the specification does not enable any person skilled in the art to which it pertains or with which it is most nearly connected, to use the invention, because the specification does not provide evidence that the claimed biological materials are (1) known and readily available to the public; (2) reproducible from the written description.

It is unclear if hybridoma DD1 which produces the antibody is known and publicly available, or can be reproducibly isolated without undue experimentation. Therefore, a suitable deposit for patent purposes is suggested. Without a publicly available deposit of the above cell line, one of ordinary skill in the art could not be assured of the ability to practice the invention as claimed. Exact replication of: (1) the claimed cell line; (2) a cell line which produces the chemically and functionally distinct antibody claimed; and/or (3) the claimed antibody's amino acid or nucleic acid sequence is an unpredictable event.

The results indicate that divergent variable region sequences, both in and out of the complementarity-determining regions, can be

folded to form similar binding site contours, which result in similar immunochemical characteristics. [FUNDAMENTAL IMMUNOLOGY 242 (William E. Paul, M.D. ed., 3d ed. 1993)]. Therefore, it would require undue experimentation to reproduce the claimed hybridoma DD1 producing the antibody. Deposit of the hybridoma would satisfy the enablement requirements of 35 USC Section 112, first paragraph. See 37 CFR 1.801-1.809.

Applicants disagree. Hybridoma DD1 is defined in the specification (page 6, lines 1 and 2) as the hybridoma described in the U.S. patent 5,334,528. Since the patent issuance, this hybridoma is in public domain. Applicants also respectfully point out that the hybridoma DD1 is known and publicly available with ATCC Accession# HB9741 as described in the Vanderlaan reference (5,429,925) cited by the Examiner.

Examiner's rejection under 35 USC 112, first paragraph, thus makes no sense. Examiner cites Vanderlaan reference and that reference clearly states in column 5, lines 47-50, that DD1 hybridoma is publicly available. Examiner even says so himself in the 103 rejection. Applicants respectfully suggest to the Examiner that he should reconsider and withdraw this rejection.

It is requested that the rejection under 35 USC 112, first paragraph, should be withdrawn.

Rejections Under 35 USC 103

Examiner maintains rejections 1-3 and 8-13 under 35 USC 103(a) as being unpatentable over Horwitz et al (Proc. Natl. Acad. Sci. USA 85:8678-8682, 1998) and further in view of Cregg et al

(Developments in Industrial Microbiology 29:33-41, 1988) and The Invitrogen 1977 Catalog (published 1/97, Yeast expression pages 14-17 and Master Catalog Amendment Notice for pPICZ vectors from 4/15/96) and Sambrook et al (Molecular Cloning, A Laboratory Manual Second Edition pages 1.85, 12.16-12.20, and 13.42-13.44, 1989) is maintained.

Applicants disagree. The fact remains that no combination of references cited by the Examiner would produce the current method depicted in the claims, as amended. The Examiner persists in combining four or more references which, individually or in combination, that is, one in view of another, would not achieve the result of the current invention.

The current method, encompassed in the currently amended claims, produces large amounts of the whole intact antibodies in amounts from about 10 milligrams to about 36 milligrams in from 12 to 108 hours (half a day to about 4.5 days). No method described before ever achieved the same results.

The closest method Examiner can come up with is the method of Horwitz, which produces 100 micrograms of antibodies in 3 days, that is, 100-360 times less than the current method and it takes six (6) times as long as the shortest time (12 hours) of the current method when at least 10 milligram of intact entire antibody is produced.

Examiner argues, without providing any evidence, that the current method is obvious because by combining Horwitz with Cregg, with the Invitrogen Catalog and with general methods described in

Sambrook et al., Molecular Cloning, A Laboratory Manual, one would expect, or have reasonable expectation, that the resulting combination could have produced a high level of intact antibody in a shorter amount of time.

To that, Applicants respond that even if, and Applicants do not agree with this reasoning, but even if the combination of all four cited references would produce a higher level of intact antibodies in a shorter amount of time, would it be really reasonable to expect to produce more than 100 to 360 times the amount as described previously by Horwitz. Such a large increase in a yield is not achieved simply by combining four unrelated references without extensive research, testing, retesting and method modifications, as detailed in the claims.

Applicants maintain that it is unreasonable for Examiner to insist that the combination of Horwitz, who is able to produce only a very small amount of the antibody in three days, using a totally different method from the current invention, with Cregg, et al., who at most teaches that a yeast is easily scaled up from shake-flask to large volume, high density cultures and that the yeast can be used for production of foreign proteins would derive the current invention. Does Cregg teach such scale-up, does he show large production of proteins, antibodies? Applicants cannot find any such teachings. All he does is describe the production of foreign proteins of noncomplex nature in general terms. There is no experimental section in this paper and no results or yields of any of his experiments are given. Applicants cannot understand how

would this paper lead to production of large amounts of antibodies which are rather complex multimeric glycoproteins and which are, even under the best of circumstance, much more difficult to produce and particularly reproduce in their entirety than the simple proteins. Cregg does not describe production of antibodies but only of simple proteins, such as enzymes β-galactosidase and alcohol oxidase. Nowhere in Cregg is there any suggestion that *P. pastoris* would be suitable for production of complex molecules such as antibodies. As a matter of fact, there is nowhere in Cregg paper any coherent description of the methodology which the authors were using and it would be very difficult, if not impossible to reproduce the method even for the simple proteins which it is suppose to produce.

Examiner produces lengthy justifications for his rejections of this application. Applicants maintain that if the invention would be truly obvious, such dissertations would not be necessary. To argue that an increase in yield of the antibody production by more than 100-360 times is obvious in view of unproven combination of four unrelated references is erroneous and unsupportable.

However, to overcome this rejection and to further distinguish the current invention from the combination of cited references, Applicants introduced the distinguishing features, that is the limitation related to the yield of production of antibodies at 10-36 mg/l in about 12 hours to about 108 hours (4.5 days) into claims.

Applicants respectfully request Examiner to reconsider this

rejection and to let the application to issue.

SUMMARY

In summary, claims 6 and 7 are cancelled and claims 1, 5, 11, 19, 20 and 21 are amended. Arguments in support of the amended claims are submitted. With these amendments and arguments, Applicant believes that the rejections of claims 1-13 and 19-21 are overcome. Notice of Allowance is respectfully solicited.

Should Examiner find that further amendments are needed, Examiner is encouraged to call the undersigned at 650-324-1677.

1-5
8-13
19-21

Respectfully submitted,

Date: February 14, 2001

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VERSION WITH MARKINGS SHOWING CHANGES

1.(Twice Amended) A method for a production of about 10-36 mg/l of a recombinant antigen-specific entire intact monoclonal antibodies[y] in about 12 hours to about 108 hours, said method comprising steps:

(a) [selecting an antigen against which the specific antibody is to be produced and] isolating, chemically synthesizing or amplifying with polymerase chain reaction (PCR) a cDNA, mRNA or genomic DNA encoding [antibody] a light [and] or heavy chain[s] of the antigen-specific antibodies and assembling the antibodies[y] cDNA encoding said [antibody] light and heavy chains of said antibodies into two separate expression cassettes, one encoding DNA for the light chain and the second encoding DNA for the heavy chain, each cassette further comprising a flanking signal DNA sequence preceded by a yeast promoter at 5' terminus and by the yeast transcription termination DNA sequence of the 3'-terminus;

(b) preparing a recombinant *Pichia pastoris* (*P. pastoris*) yeast expression vector pPICZα by restriction digestion with EcoRI and BamHI;

(c) constructing a recombinant *P. pastoris* yeast expression plasmid containing the expression cassettes of step (a);

(d) cloning the expression cassettes of step (c) into the *P. pastoris* expression vector pPICZα to generate recombinant plasmid pPICZαLH comprising expression cassettes for the light and heavy chains;

(e) transforming *Saccharomyces cerevisiae* with the recombinant plasmid by placing said expression cassettes of step (d) under the control of the AOX1 promoter fused to the DNA encoding the *Saccharomyces cerevisiae* α-mating factor signal;

(f) amplifying and isolating the recombinant plasmid;

(g) transforming *P. pastoris* spheroblasts with *Bgl*II linearized, *Not*I linearized, *Sac*I linearized, *Sal*I linearized or

*Stu*l-linearized recombinant plasmid replacing the yeast chromosomal AOX1 DNA sequence with AOX1-antibody DNA sequence containing expression cassettes of the recombinant plasmid of step (d);

(h) selectively growing the recombinants;

(i) screening yeast transformation colonies for a recombinant antibody expression;

(j) analyzing putative positive yeast clones for chromosomal integrates of the expression cassettes of heavy and light chain cDNAs;

(k) confirming the integrity of the DNA insert;

(l) inducing the recombinant antibody expression;

(m) confirming the intactness of the expression cassettes inserts with PCR and Northern blot analysis;

(n) detecting the presence of the recombinant antibody by Western blot;

(o) testing the recombinant antibody for specific antigen-antibody binding, and

(p) harvesting the antigen-specific antibody produced in steps (a) - (o) wherein said antibody is produced in quantity of 10-36 mg/l in about 12 to about 112 hours.

5. (Twice Amended) The method of claim 4 wherein the antibody cDNA encoding the light and heavy chain is isolated from a hybridoma DD1 (ATCC Accession Number HB9741) that recognizes dioxin.

11. (Twice Amended) The method of claim 9 wherein the screening of transformed colonies for antibody expression is by colony-immunoblotting [for the origin of the recombinant antibody].

19. (Twice Amended) A recombinant *Pichia pastoris (P. pastoris)* yeast expression vector containing dual expression cassettes, each cassette carrying an entire cDNA copy of immunoglobulin light [and] or heavy chain DNA and further comprising a flanking signal DNA sequence preceded by a yeast

promoter at 5'-terminus and by the yeast termination DNA sequence of the 3'-terminus, said vector useful in a method for production of a recombinant antigen-specific antibody in amounts from about 10 to about 36 mg/l in about 12 to about 108 hours.

20. (Twice Amended) An expression vector comprising *Pichia pastoris (P. pastoris)* transformed with human, mouse or humanized mouse immunoglobulin monoclonal cDNA for production of an entire recombinant antigen-specific intact antibody in amounts from about 10 to about 36 mg/l in about 12 to about 108 hours.

21. (Twice Amended) *Pichia pastoris (P. pastoris)* yeast transformed with two expression cassettes one of which carries [ying] a cDNA of a light chain of an anti-dioxin immunoglobulin [heavy] and the second of which carries a cDNA of a heavy chain of an anti-dioxin immunoglobulin, [light chain] said transformed yeast useful in the method for production of intact antibodies isolated from DD1 hybridoma (ATCC Accession Number HB9741), in amounts from about 10 to about 36 mg/l in from about 12 to about 108 hours.